A Study of the Practicality of Using an ALS Bend Magnet as the X-Ray Source for Micro Protein Crystallography.

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INTRODUCTION

Using the technique of Protein Crystallography, synchrotron sources have played a growing role in solving the most difficult and important problems in structural biology. However, it is basically difficult to obtain large crystals (~200micron) of most proteins of interest that regular protein crystallography uses. It would be much easier if protein crystallography could be carried out with micro crystals as small as 20 micron. This would advance the progress rate of structural biology significantly. Diffraction from such small samples requires the development of x-ray microprobe tools. This work describes experiments underway to optimize camera designs and experimental protocols for x-ray diffraction studies with protein micro-crystals.

The ALS development beamline 7.3.3 has been used to carry out these baseline studies. These studies have shown :-

(1) that a 40 micron x-ray beam gives acceptable background for protein crystals as small as 25 x 25 x 10 micron,
(2) that the exposure times will be very acceptable when the ALS superbend sources become available, and
(3) that radiation damage will require the use of only a few such crystals per complete data set.

Experimental

Micro Crystal Diffraction

A key objective of this work has been to establish what would be the smallest protein crystal that could be used to obtain high-quality diffraction data. Bacteriorhodopsin crystals were used for this purpose as they only rarely grow to a size as large as 100 micron; and abundant quantities of crystals in all size ranges below that are readily at hand.

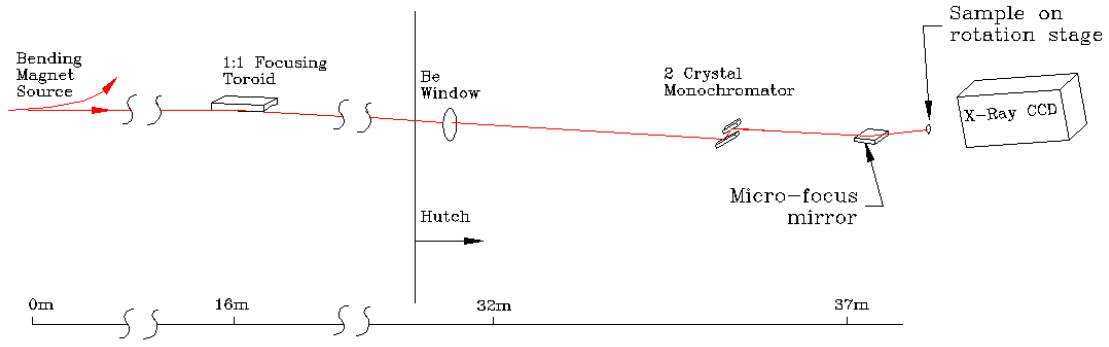

Figure 1. Schematic layout of test beamline 7.3.3 used for micro protein crystallography.

Fig.1 shows the schematic layout of the ALS test beamline 7.3.3. The mirror at 16m collects 3x0.2 mrads of radiation and focuses the light to a 300x300micron spot in the sample region. The radiation is monochromatized with a Ge(111) double crystal monochromator of bandpass ~1000. The photon energy used was 11KeV. Within the hutch there is a vertical micro focus mirror just before the sample that is able to provide additional vertical focussing such that the vertical spot size is reduced to 40 micron with a 3 mrad convergence angle. Spot size reduction in the horizontal was not possible by mirrors as the light already has a 3mrad convergence angle and this represents a maximum for micro focus mirrors at 11KeV. Slits located just before the sample allowed controlled horizontal reduction of the spot size to 40x40 micron with 3 mrad convergence angle in both planes. This convergence angle is a standard value for protein crystallography

A one-degree rotation photograph was recorded for a bacteriorhodopsin crystal that was 25 x 25 x10 micron in size. The unit cell dimensions for this crystal are 6.2nm, 6.2nm and 10.8nm. Spots were seen out to 2.3A. The exposure time was 600 seconds. The air-scatter background was at an acceptably low level, and this could be further reduced by operating with helium gas rather than nitrogen gas through the heat exchanger of the gas-stream sample cooler. For reasons of high thermal strain in the Ge(111) monochromator and the first mirror not being at the correct focus, the x-ray intensity available on this test setup at the time of these experiments was about 10-times less than it could be. Also of note is that the intensity with an ALS superbend magnet as the source will be higher by an additional factor of ~10. This would indicate that on a superbend with a correctly engineered beamline and optics, diffraction patterns could be taken from bacteriorhodopsin crystals as small as $(20\mu m)^3$ with 6 second exposures for 1 degree of rotation. This would mean that data collection times for micro crystals would no longer be a limitation.

Radiation Damage

In separate experiments with larger crystals and a larger x-ray beam, we have also examined the degree to which radiation damage limits the accumulated x-ray exposure that can be tolerated. Data were collected over a 10-degree wedge, in 1-degree steps; the goniometer was returned to the original setting and data were collected over the same 10-degree wedge. The process was repeated until substantial damage could be seen. The experiments used crystals that diffracted initially to about 2.1A resolution. After an accumulated exposure of 10^{10} photons/micron^2, one could no longer see diffraction spots at a resolution higher than about 2.5A, and reflections that remained at lower resolution were much weaker than in the first cycle. While measurements were not extended beyond an exposure of 10^{10}photons/micron^2, it is unequivocally clear that crystals would no longer be useful for data collection after a 10-fold higher exposure, i.e. 10^{11} photons/micron^2. This conclusion had already been drawn by Gonzales and Nave, using exposure of protein crystals to a white-source beam at Daresbury.

The 600-second exposure referred to earlier to record the diffraction pattern from the ~20 micron crystal corresponded to less than 10^9 photons/micron^2. Thus we estimate that one could collect at least 10 degrees of data before radiation damage would require changing the crystal. A full data set, i.e. 90 degrees of data, could then be collected with fewer than 10 such micro crystals. More generally, the following rules-of-thumb are expected to apply to data collection from protein microcrystrals:

CONCLUSIONS

1. Routine data collection will become possible on crystals that are 30 to 40 micron in size. This option should be especially valuable in those cases where efforts to improve crystal size are not immediately productive. The cost of prolonged efforts to increase crystal size will no longer be justified.
2. The small crystal size will allow one to diffuse a reactant (or small-molecule ligand) into the protein much more rapidly than would be the case for crystals of the "standard" size. The use of very small protein crystals will greatly increase the number of cases in which reactants can be loaded within the enzyme to relatively high occupancy at a rate faster than the reactant is processed by the enzyme. Also one will not have to use the extremes of low temperature that would otherwise be necessary to accomplish this.
3. The small crystal size may also make it possible to freeze crystals without the need to soak in a cryotectant, as is already known to be true for the micro crystals that are used in electron cryo-microscopy. Valuable time will be gained if one can bypass the search for conditions that preserve crystal quality and still allow crystal freezing.

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